

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
16 February 2006 (16.02.2006)

PCT

(10) International Publication Number
WO 2006/016372 A1

(51) International Patent Classification:
A01N 1/02 (2006.01) F25D 3/00 (2006.01)

(74) Agent: REINHOLD COHN AND PARTNERS; P.O.Box
4060, 61040 Tel Aviv (IL).

(21) International Application Number:
PCT/IL2005/000876

(22) International Filing Date: 14 August 2005 (14.08.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/600,804 12 August 2004 (12.08.2004) US

(71) Applicant (for all designated States except US): I.M.T.
INTERFACE MULTIGRAD TECHNOLOGY LTD.
[IL/IL]; 3 Hamazmera Street, 70400 Ness Ziona (IL).

(72) Inventors; and

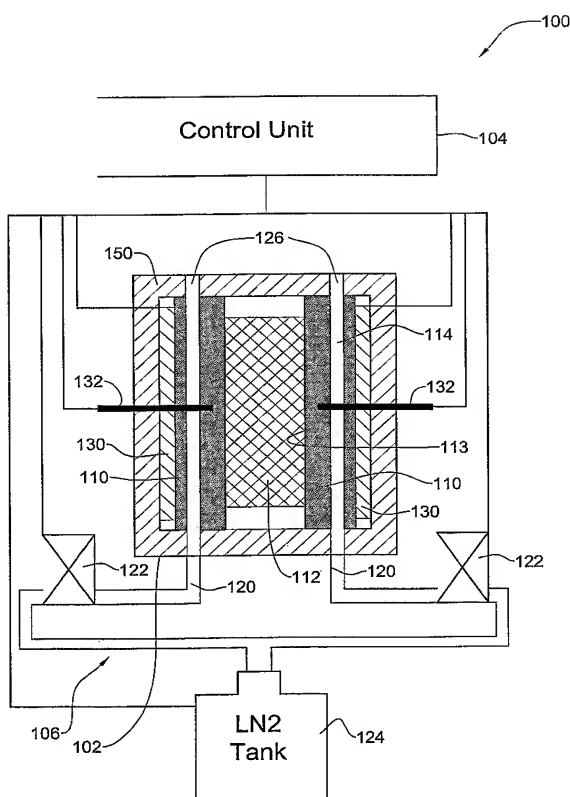
(75) Inventors/Applicants (for US only): SHAHAM, Ginadi
[IL/IL]; 15 Nophar Street, 81207 Yavneh (IL). DAMARI,
Udi [IL/IL]; 14 HaCarmel Street, 55900 Ganey Tikva (IL).
RUSSO, Benny [IL/IL]; 3 HaEtgar Street, 39120 Tirat
Carmel (IL).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE,
KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,
MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ,
OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL,
SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC,
VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR FREEZING OR THAWING OF A BIOLOGICAL MATERIAL



(57) Abstract: The temperature of a biological material is changed from a first temperature to a second temperature within a time period, one of the said first or second temperature being above freezing temperature and the other being below freezing temperature, by placing the biological material in tight contact with at least one, preferably between two heat exchangers, and controlling the temperature in at least one of said heat exchangers such that a freezing temperature front propagates in said material away from at least one of the two heat exchangers.

WO 2006/016372 A1



Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

- 1 -

METHOD AND APPARATUS FOR FREEZING OR THAWING OF A BIOLOGICAL MATERIAL

FIELD OF THE INVENTION

This invention relates to a method and an apparatus for freezing or thawing of a biological material.

LIST OF REFERENCES

5 The following references are considered to be pertinent for the purpose of understanding the background of the present invention:

1. US Patent No. 5,873,254;
2. WO 03/056919;
3. WO 03/020874;
- 10 4. US 6,337,205 (Wisniewski);
5. US 5,863,715 (Rajotte).

BACKGROUND OF THE INVENTION

15 In freezing of biological material, two freezing stages are recognized: nucleation and crystallization. In the first stage ice nucleation occurs in the solution outside the cells. In order to minimize cellular damage, it is critical to control during this stage (nucleation) both the interface velocity of the cold front and the direction of thermal gradient within the object. Normally, in some biological materials (e.g. blood, cell suspensions, plasma, semen and other liquid samples) the best survival is obtained when the freezing rate at this stage is relatively rapid (10°C/min or more). In other cases (e.g. organs or organ fragments), it is
20 accepted that a slow freezing rate at this stage (0.5°C/min or less) would improve freezing.

 The next stage is that of crystallization, an exothermic process that produces latent heat within the frozen material, causing a period of time when the biological material remains isothermal, or even experiences an increase in temperature: latent heat exudes

- 2 -

from the biological material and thus, although the material is being cooled no temperature change is observed or the temperature may even rise. This in turn causes spontaneous freezing and thawing cycles which are hazardous to the biological material.

5 Permitting osmosis of water out of the cells at this stage would reduce damage to the cells, and the increase of intracellular concentration would cause the cells to vitrify rather than freeze. This is affected by the rate of freezing, and thus, in order to optimize the biological material's survival of this stage control of the rate of freezing is important. The optimal rate depends on the type and composition of the biological material being frozen.

10 In addition to the above, cryopreservation of material having a large volume (e.g. tissues, organs or portions thereof) is associated with heat transfer and mass problems that are not associated to the same extent with cryopreservation of isolated cells. For example, in conventional freezing methods, ice grows at an uncontrolled velocity and morphology and may disrupt and kill cells by mechanical destruction of the tissue architecture. Due to
15 the large size of macroscopic material, large uncontrolled thermal gradients may develop from the surface of the sample to its interior.

One method that was devised to allow freezing biological material of a large volume is disclosed in US 5,863,715. In this patent, the biological material is placed in a flexible container, such as a bag. The bag is then flattened in a holder that maintains an
20 essentially constant cross-sectional area of the bag in order to minimize thermogradients. The holder is then cooled along with the bag contained therein.

It is well established that directional freezing, a process in which a cold front propagates in a controlled manner through the frozen object, improves the chances of biological material to survive freezing and thawing. In this process a temperature pattern
25 (or gradient) is established in the object being frozen to form a propagation cold front within the object, resulting in improved chances of survival.

A successful method of directional freezing is disclosed in US Patent No. 5,873,254. In this patent, a freezing apparatus is used to establish a laterally varying thermal gradient and the biological sample is moved along the thermal gradient at a
30 controlled velocity. Additional methods were developed in order to improve the freezing of large volume objects. For example, WO 03/056919 discloses freezing biological samples via an isothermal stage, wherein the temperature is changed until temperature of the sample in an outer zone equals intermediate temperature and changing temperature

- 3 -

until the majority of the sample is in a final temperature. This method may be used in conjunction with directional freezing but is not limited thereto. Another process is disclosed in WO 03/020874 in which the biological sample is agitated during its migration along a thermal gradient.

5 A method for cryopreservation of a biopharmaceutical is disclosed in US 6,337,205. The sample to be frozen is inserted into special oblong vials that have special appendages, termed "*ice crystal-nucleating structures*", situated at the opposite ends of the vial's oblong cross-section. The vials are placed within a compartment of a cryopreservation apparatus, said compartment containing a cryopreservation fluid. A
10 freezing front is then generated at one of the walls of the apparatus that is adjacent to one of the appendages, and propagates through the cryopreservation fluid. Due to the special shape of the appendage, nucleation begins at the appendage, and thus the cold front propagates within the sample in a direction that is away from the cooling wall and along the oblong cross section of the vial. In an alternative disclosed in US 6,337,205, two cold
15 fronts may be generated in the compartment, in opposing directions, by opposing walls of the apparatus. In this method, the freezing of the sample is achieved indirectly, in the sense that the cooling wall of the apparatus cools the cryopreservation fluid, which in turn cools the vial (and the sample within it).

SUMMARY OF THE INVENTION

20 The present invention is based on the realization that freezing and/or thawing of a biological material, for example an organ to be preserved for subsequent transplantation, may be carried out with minimal damage by placing the biological material in tight contact with at least one, preferably between at least two heat exchangers, and controlling the temperature so as to yield a gradual temperature change in the biological material that will
25 give rise to directional freezing. More particularly, this is achieved by one or more controlled thermal gradients within the material, inflicted by the heat exchangers, such that a freezing temperature front propagates within the material, so as to gradually freeze the entire material in a directional manner.

 According to a first aspect of the invention there is provided a method for changing
30 the temperature of a biological material from a first temperature to a second temperature within a time period, one of the said first or second temperatures being above freezing

- 4 -

temperature and the other being below freezing temperature, comprising: placing the biological material tightly in contact with at least one, preferably in tight contact between at least two, heat exchangers and inducing a heat exchange scheme comprising controlling the temperature in at least one of said heat exchangers such that a freezing temperature front propagates in said material away from said heat exchangers

According to another aspect there is provided an apparatus for changing the temperature of a biological material from a first temperature to a second temperature, one of the said first or second temperature being above freezing temperature and the other being below freezing temperature, the apparatus comprising: a heat exchange unit comprising at least one heat exchanger for placement in contact with the biological material, one or more of the at least one heat exchanger being equipped with a temperature control arrangement including one or both of a heating and a cooling arrangement, at least one of said heat exchangers being equipped with a cooling arrangement; and a control unit for inducing a temperature changing operational sequence comprising controlling temperature of at one or more of the least one heat exchanger such as to yield a freezing temperature front that propagates within said material away from at least one of said heat exchangers, whereby temperature of the material changes from said first to said second temperature.

At times the heat exchange unit comprises at least two heat exchangers situated opposite one another with a space therebetween for accommodating the biological material, each of said heat exchangers being equipped with one or both of a heating and a cooling arrangement, at least one of said heat exchangers being equipped with a cooling arrangement

Said controlling typically involves gradually changing the temperature of at least one, typically more than one, of the heat exchangers.

As will be appreciated, during operation, preferably one but usually more than one heat exchangers will have a temperature below freezing in at least part of the temperature changing operational sequence. Additionally, while in some embodiments of the invention the heat exchangers may have an essentially constant temperature throughout the temperature changing operational sequence, in other embodiments of the invention the temperature of one or more of the heat exchangers may be changed, e.g. gradually, during the temperature changing operational sequence.

- 5 -

While changing of the temperature of the biological material, in accordance with the invention, a temperature gradient is formed within the biological material. This requires that during operation at least one of the heat exchangers will have a temperature below freezing point. Consequently, a portion of the biological material adjacent such heat exchanger freezes first. The interface between a frozen portion and a non frozen portion in the biological material creates a "*freezing temperature front*", which may then gradually propagate in said material as other portions of the material gradually freeze. The rate of propagation may be controlled by the temperature differential between heat exchangers and also by a change in temperature in one or both of the heat exchangers, or by changing the distance between the two heat exchangers. It should be noted that a freezing temperature front also occurs while thawing during gradual transition of the biological material from frozen to a thawed state, and such front also propagates in the material in a similar manner to that described above. Similarly, during thawing, the rate of propagation of the freezing temperature front may be controlled by a change in the temperature of one or both of the heat exchangers.

In accordance with one embodiment of the invention a temperature differential between the two heat exchangers is maintained at least during part of the temperature change period, such that the freezing temperature front propagates from one side, adjacent one of the heat exchangers, to the other side of the biological material adjacent the other heat exchanger.

In accordance with another embodiment, the temperature of both heat exchangers is maintained at essentially the same temperature such that freezing temperature front propagates from the periphery of the biological material, adjacent said heat exchangers, towards the interior of the material, somewhat in between the two heat exchangers. While in the former embodiment, the freezing will be in one direction throughout the entire biological material, in the latter embodiment the freezing will in fact be bi-directional.

In accordance with an embodiment of the invention a plurality of heat exchangers are provided for contacting the biological material from a plurality of directions. Thus, in accordance with this embodiment, a heat exchange scheme may be induced, with a plurality of freezing temperature front propagate within the biological material towards its interior. In a specific embodiment a plurality of heat exchangers for essentially surrounding the biological material are provided. In the latter embodiment, a

- 6 -

heat exchange scheme in which a freezing temperature front propagates to the biological material's interior from all peripheral portions thereof. This will give rise to multidirectional freezing from the periphery towards the material's interior.

As may be appreciated, at times the at least two heat exchangers are not independent bodies but rather may be part of one heat exchange device. For example, in one embodiment the at least two heat exchangers may be integrally formed with a main body that holds the temperature control arrangement.

By another exemplary embodiment, the heat exchangers are formed as a tubular body, which may have a cylindrical, elliptical, oval, polygonal or any other suitable cross-sectional shape. Such a tubular heat exchange body is adapted to encase the biological material during temperature change according to the invention.

A plurality of heat exchangers may be so configured such that when brought together they define a tubular heat exchange structure. By a non-limiting example, two heat exchangers that are trough-shaped, when brought together form a tubular heat exchange structure. Additional heat exchangers may be provided in for insertion into the open ends of the tubular structure, to ensure tight engagement of the biological material with the heat exchangers. Such additional heat exchangers may be provided also in the case of the "another exemplary embodiment" described above.

In accordance with one embodiment of the invention more than one, and preferably all of the heat exchangers comprise or are associated with a cooling arrangement for cooling the internal, biological material-facing surface thereof.

In accordance with another embodiment, at least one, and preferably all of the heat exchangers comprise or are associated with a heating arrangement for heating the internal, biological material-facing surface thereof.

The harvested organ may typically be first cooled from a harvested temperature, typically about 37°C to a lower temperature, still above freezing, e.g. a temperature within the range of 2-6°C. This may be carried by routine refrigeration methods. Thereafter such harvested biological material may be further cooled, by the method and/or apparatus of the invention to a lower temperature, e.g. about -20°C to -80°C, at times to, a temperature in the range of -30°C to -70°C, and typically at a temperature within the range of -40°C to -60°C. Thereafter the frozen biological material may be

- 7 -

further cooled, e.g. by the use of liquid nitrogen to a temperature of about -196°C and stored at that temperature.

Thawing of a biological material frozen in accordance with the invention may be achieved by conventional means or may also be carried out in accordance with the teaching of the invention. This thawing method may be applied to frozen biological material that was not frozen by the method of the present invention. Typically, the temperature is first gradually increased to a temperature still below freezing and then the biological material is further warmed to a temperature of a few degrees above freezing, which may be carried out in accordance with the teaching of the present invention.

In some embodiments of the invention, particularly in case of the need to change the temperature of a biological material which has non homogenous thermal properties, e.g. and organ with different tissue types, it may be desired to control the temperature of the heat exchanger so as to have somewhat different temperatures at different zones of the heat exchanger that are in contact with different portions of the biological material, e.g. different portions of the harvested organ intended for preservation. Thus, heat exchangers may be equipped, in accordance with some embodiments of the invention, with two or more independent cooling and/or heating arrangements at different regions of the heat exchanger to yield different temperatures at different regions thereof.

Many options are known in the art that enable imposing a different temperature regime on a single heat exchanger. This includes constructing the heat exchanger from distinct blocks, each having a separate temperature control (and optionally each having a sensor) and separate coolers and/or heaters. Alternatively a single heating or cooling unit may be configured to generate a predefined temperature gradient by forcing the cooling fluid to flow from one side to the other (instead of bi-directional flow), causing a gradient which would be dependent on the geometry of the cooling block and of the cooling conduits. For example, a cooling fluid conduit may be configured such that it would be relatively far from the biological material facing surface when entering a heat exchanger, and grow closer to the biological material facing surface until exiting the heat exchanger at a place near the biological material facing surface. Yet another option is using several heaters or coolers, each controlled separately such that different temperatures would be imposed in different locations in a single block.

- 8 -

In accordance with one embodiment of the invention, the cooling arrangement includes conduits for cooling fluids, which conduits are associated with or are formed within the heat exchangers. Such conduits are in flow communication with a cooling fluid reservoir, typically through flow control valving means. The heating arrangement
5 may also involve such conduits, which may be the same or different than the conduits used for the cooling fluid. By controlling the rate of flow and/or the temperature of the fluid that enters the conduits, the temperature of the heat exchangers may be controlled. The cooling fluid is typically liquid nitrogen. An example of a heating fluid is water or alcohol.

10 In accordance with another embodiment, the heating arrangement includes electric heating modules.

The heat exchange unit is typically insulated from the environment, so as to minimize heat loss and improve heat transfer to or from the biological material. The insulating material may comprise Styrofoam, glass wool, cellulose wool, ceramic
15 foams, polyethylene, vacuum, and generally in any type of insulation known *per se*.

In order to improve conduction of heat to or from the biological material, particularly in case of an organ of an irregular shape, at least one of the heat exchangers may be displaceable to yield a better contact between the heat exchangers and the biological material or ensure relatively tight fitting of the biological material into the
20 space formed between the two opposing heat exchangers.

It should be noted, that the two opposing heat exchangers may, under some embodiments, be kept always parallel to one another. However, it is clear that under other embodiments, at least one of the heat exchangers may be tilted to ensure better contact with the biological material, whereby during operation the two heat exchangers
25 will not be parallel to one another.

In accordance with other embodiments of the invention, the heat exchangers may have an irregular shape, for example, so as to fit the external contours of the biological material, e.g. the external contours of a heart, a kidney, etc. Also, in accordance with embodiments of the invention, there may be more than two heat
30 exchangers, two or more on one side of the biological material and two or more on the other. Both these embodiments constitute different ways for achieving good contact between the heat exchangers and the biological material, particularly such having an

- 9 -

irregular shape (e.g. a harvested organ), thereby ensuring efficient temperature exchange with the biological materials.

At times, use may be made with heat conducting fitting members that are placed between a heat exchanger and the organ and serve as a bridge for conducting heat or
5 cold between the heat exchanger and the surface of the biological material.

At times, particularly for the purpose of ensuring sterility, the biological material may be included within a chamber or a bag. In case the container is a rigid container (e.g. a vial), it may example, be inserted into a liquid that transfers the heat or cold from the heat exchanger to the container (and then from the vial to the container to the biological
10 material, directly or through a small amount of a cryopreservation solution). Preferably, the container is held in tight contact with at least one or two heat exchangers, which contact is direct contact, such that heat is conducted from (or to) the heat exchanger directly to (or from) the wall of the container (with the possible exception of a thin film such as liquid nitrogen vapor).

15 The apparatus may comprise one or more temperature sensors for sensing the temperature of the heat exchangers or that of the biological material.

The control unit may comprise a dedicated computer or external desktop or laptop computer or PLC (Programmable Logic Controller). It may also comprise a user interface allowing a user to control or override the pre-set temperature regime.

20 In addition, in some cases it may be desirable to provide additional information to the control unit (information such as freezing temperature front propagation feedback). This information may be used for example as feedback for control of operation and also for quality assurance of the resultant temperature change of the biological material. The sensor reading may also allow the control unit and/or user to
25 adapt the temperature regime to the actual changes within the biological material. Finally, the data so collected may be stored in any form (such as digital data or printed documentation) for any use, including research and development.

Thus, in addition to temperature sensors the system might include additional sensors such as:

30 1. One or more CCD cameras that may be used for observation of the biological material and crystals formed therein;

2. One or more temperature sensors (e.g. a thermocouple or infrared camera or detector) at one or more locations within the biological material, that may be used to

- 10 -

record the temperature pattern at any time and the changes in temperature during operation;

3. One or more electrical resistance (impedance) measuring units that allow detection of changes within the biological material during operation.

5 Finally, ultrasound may also be used to follow the freezing temperature front propagation inside the biological material. In such case an ultrasound transmitter may be used, for example within the chamber, and the propagation of the interface (cold front) may be monitored by ultrasound readings as known in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

10 In order to understand the invention and to see how it may be carried out in practice, embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawing, in which:

Fig. 1 is a schematic cross sectional view of a apparatus according to one embodiment of the invention.

15 DETAILED DESCRIPTION OF THE INVENTION

 The apparatus and method of the present invention are for changing the temperature of a biological material past a freezing point for either freezing a biological material, particularly a harvested organ intended for subsequent transplantation, or for thawing a biological material, e.g. for subsequent transplantation of a preserved organ in
20 an organ recipient. The controlled freezing in accordance with the invention gives rise to a control, directional ice crystal generation in the organ.

 Reference is first being made to Fig. 1 which is a schematic representation of an apparatus in accordance with the invention generally designated **100**. The main components of the apparatus include a heat exchange unit generally designated **102**, a
25 control unit **104** and a cooling liquid supply system generally designated **106**.

 A heat exchange unit **102** includes, in this specific embodiment, two heat exchangers **110** defining a space between them **112** for accommodating the biological material to be frozen or thawed, which is typically a harvested organ or liquid biological sample (such as blood or semen) intended for cryopreservation for subsequent use for in
30 vitro purposes or in transplanting in an organ recipient. The heat exchangers are typically made of material with high thermal conductivity, e.g. a metal such as brass,

- 11 -

gold, gold plated brass, and others. Occasionally the surface of the heat exchanger may be coated by a thin layer made of polymeric material, glass, etc.

In the apparatus schematically illustrated in Fig. 1, two opposing heat exchangers are included. However, as already pointed out above, it is possible also for the apparatus to have three or more heat exchangers to permit the achievement of complex heat exchange patterns, for improved heat exchange contact with irregularly shaped biological materials, etc. Furthermore, according to some embodiments of the invention, the heat exchangers are displaceable to ensure relatively tight attachment to the biological material (e.g. to tilt at least one of them thus changing the shape of space 112 or move at least one of them thus changing the size of space 112).

Heat exchangers 110 may be positioned horizontally, vertically, or in any other suitable alignment. They may have an essentially flat internal, material facing surface 113 or may have an irregular shape, e.g. concave or convex, to permit better association with external contours of the biological material to be frozen or thawed by the apparatus.

For cooling, heat exchangers 110 are provided with conduits 114 that are linked to cooling system 106 through tubings 120. Cooling system 106 comprises flow control valves 122, disposed within tubings 120 and a cooling fluid reservoir 124, with the cooling fluid being typically liquid nitrogen, although other cryogenic fluids may also of course be used.

Illustrated herein is a single conduit 114 in each of heat exchangers 110 although as may be appreciated, the heat exchanger may be equipped with more than one conduit. Also, independent valves may be provided for different conduits, for example, for achieving different temperature at different regions of the heat exchanger.

In accordance with one embodiment, spent cooling fluid is discharged to the atmosphere through an exhaust 126. Alternatively, it is also possible to have a recycling arrangement whereby spent fluid is cooled again and returned back to reservoir 124.

A heat exchange unit further includes electrical heating modules 130 which are in tight association with the heat exchangers. Thus, heat exchangers may be either heated or cooled or through a combination of cooling and heating, a fine temperature control may be achieved.

The heat exchange unit is typically insulated by means of insulator 150.

- 12 -

The apparatus also comprises temperature sensors 132, linked to controlled unit 104. Two temperature sensors are shown in this schematic illustration, although it may be appreciated that more temperature sensors may be included, e.g. different sensors at different zones of the heat exchangers, sensors for sensing the temperature of the biological material, etc. Control unit 104 is also linked to electric heating elements 130, valves 122 and cooling reservoir 124.

Operation of an Apparatus

In operation the temperature the heat exchangers is changed to an initial temperature. At that time a biological material is placed within the apparatus such that the biological material (or the container holding the biological material) is held tightly by the heat exchangers. This may be achieved for example by moving at least one of the heat exchanger closer to the other, with the biological material sandwiched between them, but taking care not to damage the biological material. Preferably the biological material is placed in the apparatus at a predetermined initial temperature, which may be different from that of the heat exchangers of the apparatus. Once the biological material is in place, the temperature of at least one of the heat exchangers of the apparatus is changed for a period of time. As detailed below, the pattern and rate of temperature change may be preset or may be modified during operation in response to processes in the biological material.

It is well established that different biological samples require different freezing protocols in order to survive freezing and thawing and remain biologically active. Many such protocols are known in the art. Examples for such protocols are given in US 5,873,254. For example: freezing of semen may begin at a slow rate (e.g. 1°C/minute) from a temperature of 30 °C. to 4°C. Next, a faster rate (30 °C/minute) would be used until the semen reaches about -50 °C, when crystallization ceases. To achieve this protocol, the apparatus may be set such that a sensor within the biological material (in this example semen) would dictate the changing of rate of temperature change or a CCD camera or ultrasound transmitter would be used to detect the time when a change of rate is desired (e.g. after lipid phase transition or crystallization are complete) and then change the heat exchanger temperature regime, either automatically or by a user.

The rate of temperature change in the biological material would be proportional to the temperature difference between the material and the heat exchangers, the rate of change in the heat exchangers' temperature and the thermal properties of the biological

- 13 -

material. Thus the heat exchangers' temperature regime may be set according to calculations based on the above parameters, or according to trial and error experimentation, or on both.

The time upon which a change in the temperature regime of any heat exchanger (or portion of a heat exchanger) would be affected may be set according to any calculable or observable parameter. Accordingly the control unit may be configured to change the heat exchangers' temperatures upon reaching of certain time or temperature thresholds. Some non-limiting examples are:

- (a) A time-dependent change, namely a change that occurs within a given time after operation began;
- (b) A temperature-dependent change that begins at a time when the heat exchanger's or the biological material's measured temperature reaches a specific temperature or is within a pre-defined temperature range; or
- (c) A temperature profile-dependent sequence that is initiated at a time when a certain temperature gradient is achieved within the biological material.

Alternatively, the change of temperature regime may be in accordance with a process observed within the biological material, such as upon the beginning or termination of any one of the following processes: seeding, lipid phase transition, nucleation, crystallization, glass transition. The change of a regime may be effected automatically by the controller through feedback from one or more sensors of any kind. Alternatively this change of regime may be manipulated manually by the user in real time in accordance with said sensor readings.

Finally, as noted above, the temperature regime of each of the heat exchangers may be different from that of the other, and the temperature difference between them may change during operation. This difference may also be preset, but may also be changed during operation, according to the sensor feedback from the heat exchangers or the biological material or according to any of the abovementioned processes that are observed within the biological material.

It is appreciated that the above discussion regarding operation of an apparatus according to a specific, non-limiting embodiment of the present invention, applies, *mutatis mutandis*, also to the method of the invention, even when an apparatus according to the invention is not used.

- 14 -

Those skilled in the art will readily appreciate that various modifications and changes can be applied to the embodiments of the invention as hereinbefore exemplified without departing from its scope defined in and by the appended claims.

CLAIMS:

1. A method for changing the temperature of a biological material from a first temperature to a second temperature within a time period, one of the said first or second temperatures being above freezing temperature and the other being below freezing temperature, comprising:
5 placing the biological material tightly in contact with at least one heat exchanger and controlling the temperature in one or more of the at least one heat exchanger such that a freezing temperature front propagates in said material away from said heat exchanger.
- 10 2. A method according to Claim 1, wherein said controlling comprises gradually changing the temperature of at least one of the heat exchangers.
3. The method of Claim 1 or 2, for freezing said biological material.
4. The method of any one of Claims 1-3, wherein said biological material is contained in a container.
- 15 5. The method of any one of Claims 1-4, wherein said biological material is an organ for transplantation.
6. A method of any one of Claims 1-5, wherein said biological material is placed between at least two heat exchangers.
7. The method of Claim 6, wherein there is a temperature difference between the
20 two heat exchangers in at least one stage during said time period.
8. The method of Claim 7, wherein the freezing temperature front propagates within the biological material in a direction from one heat exchanger to the other.
9. The method of Claim 6, wherein the temperature of the two heat exchangers is essentially the same and said freezing temperature front propagates from zones of the
25 material adjacent the two heat exchangers to the material's interior.
10. The method of any one of the preceding claims, wherein the one or more heat exchangers essentially surrounds the biological material.
11. An apparatus for changing the temperature of a biological material from a first temperature to a second temperature, one of the said first or second temperature being
30 above freezing temperature and the other being below freezing temperature, the apparatus comprising:

- 16 -

a heat exchange unit comprising at least one heat exchanger for placement in contact with the biological material, one or more of the at least one heat exchanger being equipped with one or both of a heating and a cooling arrangement, at least one of said heat exchangers being equipped with a cooling arrangement; and

5 a control unit for inducing a temperature changing operational sequence comprising controlling the temperature of one or more of the at least one heat exchanger such as to yield a freezing temperature front that propagates within said material away from at least one of said heat exchangers, whereby temperature of the material changes from said first to said second temperature.

10 12. The apparatus of Claim 11, wherein said controlling comprises gradually changing the temperature of one or more of the at least one heat exchanger.

13. The apparatus of any one of Claims 11-12, wherein the at least one heat exchanger comprises a cooling arrangement for cooling the internal, biological material facing surface of said heat exchanger.

15 14. The apparatus of any one of Claims 11-13, wherein one or more of the at least one at least one heat exchanger comprises a heating arrangement for heating the internal, biological material facing surface of said heat exchangers .

15. The apparatus of any one of Claims 11-14, wherein one or more of the at least one at least one heat exchanger comprises a cooling and a heating arrangement for
20 respective cooling and for heating the internal, biological material facing surface of said heat exchanger.

16. The apparatus of any one of Claims 13 and 15, wherein said cooling arrangement comprises one or more cooling fluid conduits in flow communication with a cooling fluid reservoir.

25 17. The apparatus of Claim 14 or 15, wherein said heating arrangement comprises an electric heating module.

18. The apparatus of any one of Claims 11-17, wherein said heat exchange unit is thermally insulated.

19. The apparatus of any one of Claims 11-18, comprising at least one or more
30 temperature sensors for monitoring temperature of one or more of said at least one heat exchanger and said biological material.

- 17 -

20. The apparatus of any one of Claims 11-19, wherein the biological material is held within a container.

21. The apparatus according to any one of Claims 11-20, wherein the heat exchange unit comprises at least two heat exchangers situated opposite one another with
5 a space therebetween for accommodating the biological material, each of said heat exchangers being equipped with one or both of a heating and a cooling arrangement, at least one of said heat exchangers being equipped with a cooling arrangement.

22. The apparatus of Claim 21, wherein during at least some of the operational sequence there is a temperature difference between said two heat exchangers.

10 23. The apparatus of any one of Claims 21-22, wherein the two heat exchangers are displaceable.

24. The apparatus of any one of Claims 21-23, wherein the control unit is adapted to control the temperature of each of said two heat exchangers independently.

1/1

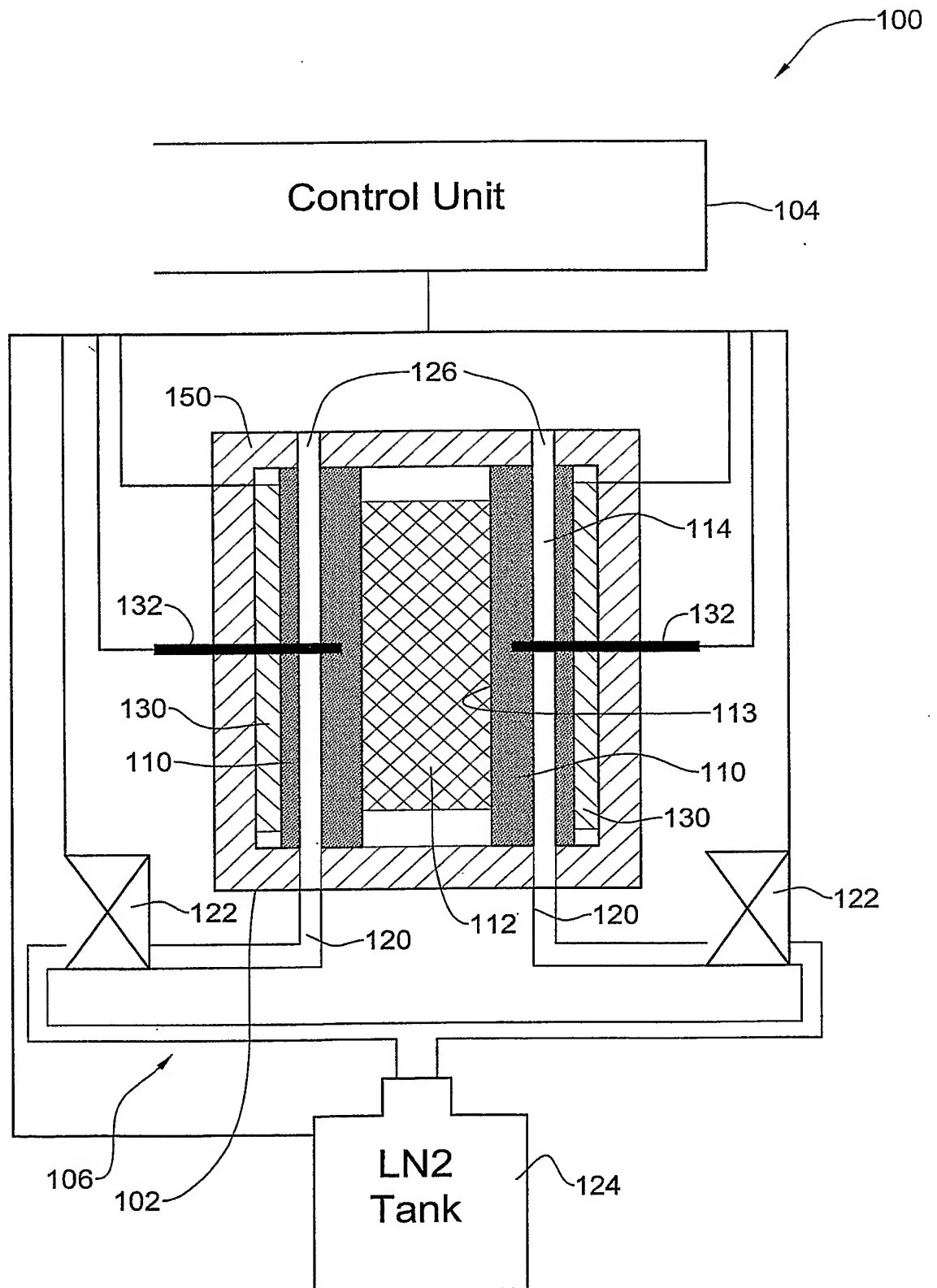


FIG. 1

INTERNATIONAL SEARCH REPORT

PCT/IL2005/000876

A. CLASSIFICATION OF SUBJECT MATTER
A01N1/02 F25D3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01N F25D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/129003 A1 (VOUTE NICOLAS ET AL) 8 July 2004 (2004-07-08) the whole document	1-24
X	US 6 453 683 B1 (WISNIEWSKI RICHARD ET AL) 24 September 2002 (2002-09-24) the whole document	1-24
X	US 5 873 254 A (ARAV ET AL) 23 February 1999 (1999-02-23) cited in the application the whole document	1-24
X	WO 03/056919 A (I.M.T. INTERFACE MULTIGRAD TECHNOLOGY LTD; ARAV, AMIR) 17 July 2003 (2003-07-17) cited in the application the whole document	1-24
	----- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

14 December 2005

Date of mailing of the international search report

21/12/2005

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Bertrand, F

INTERNATIONAL SEARCH REPORT

PCT/IL2005/000876

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/055456 A (INTEGRATED BIOSYSTEMS, INC; VOUTE, NICOLAS; WISNIEWSKI, RICHARD; LEE,) 1 July 2004 (2004-07-01) cited in the application the whole document	1-24
X	WO 01/50852 A (INTEGRATED BIOSYSTEMS, INC) 19 July 2001 (2001-07-19) cited in the application the whole document	1-24

INTERNATIONAL SEARCH REPORT

PCT/IL2005/000876

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 2004129003	A1	08-07-2004	NONE		
<hr/>					
US 6453683	B1	24-09-2002	CA	2446938 A1	28-11-2002
			CN	1541323 A	27-10-2004
			EP	1389292 A1	18-02-2004
			JP	2004527720 T	09-09-2004
			WO	02095306 A1	28-11-2002
			US	2002177119 A1	28-11-2002
<hr/>					
US 5873254	A	23-02-1999	AU	4244797 A	26-03-1998
			EP	0925475 A1	30-06-1999
			WO	9810231 A1	12-03-1998
<hr/>					
WO 03056919	A	17-07-2003	AU	2003207952 A1	24-07-2003
			EP	1476013 A2	17-11-2004
<hr/>					
WO 2004055456	A	01-07-2004	AU	2003297056 A1	09-07-2004
			CA	2509037 A1	01-07-2004
			EP	1570217 A1	07-09-2005
<hr/>					
WO 0150852	A	19-07-2001	AT	267519 T	15-06-2004
			CA	2390917 A1	19-07-2001
			DE	60103498 D1	01-07-2004
			DE	60103498 T2	30-06-2005
			DK	1246524 T3	27-09-2004
			EP	1246524 A2	09-10-2002
			ES	2220778 T3	16-12-2004
			JP	2003519513 T	24-06-2003
<hr/>					